

## THE EFFECTS OF ACCLIMATION TEMPERATURE ON THE DYNAMICS OF CATECHOLAMINE RELEASE DURING ACUTE HYPOXIA IN THE RAINBOW TROUT *ONCORHYNCHUS MYKISS*

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### Summary

The response of cannulated rainbow trout (*Oncorhynchus mykiss*) to acute hypoxia was studied in fish acclimated to two temperatures (5 and 15 °C). Blood/water respiratory variables and plasma catecholamine levels were measured before and 15 min after exposure to hypoxic water varying between 4.0 and 10.7 kPa (30–80 mmHg) oxygen partial pressure ( $P_{\text{wO}_2}$ ). Arterial blood  $P_{\text{O}_2}$  ( $P_{\text{aO}_2}$ ) and oxygen content ( $\text{CaO}_2$ ) fell during hypoxia in a similar manner at both temperatures, although the changes in  $\text{CaO}_2$  were often more pronounced in the fish acclimated to 15 °C. Regardless of acclimation temperature, plasma catecholamine levels were consistently elevated at  $P_{\text{wO}_2}$  values below 8.0 kPa (60 mmHg); the largest increases in plasma catecholamine levels occurred below  $P_{\text{wO}_2}$ =5.3 kPa (40 mmHg). Adrenaline was the predominant catecholamine released into the circulation. Adrenaline was released at  $P_{\text{wO}_2}$  values of 8.0 kPa or below, whereas noradrenaline was released at  $P_{\text{wO}_2}$  values of 6.7 kPa or below.

The construction of *in vivo* oxygen dissociation curves demonstrated an obvious effect of acclimation temperature on haemoglobin (Hb) oxygen-affinity; the  $P_{50}$  values at 15 °C and 5 °C were 3.6 kPa (26.7 mmHg) and 1.9 kPa (14.0 mmHg), respectively. At 15 °C, catecholamines were released into the circulation abruptly at a  $P_{\text{aO}_2}$  threshold of 4.6 kPa (34.5 mmHg) while at 5 °C the catecholamine release threshold was lowered to 3.3 kPa (24.5 mmHg). The difference in the  $P_{\text{aO}_2}$  catecholamine release thresholds was roughly equivalent to the difference in the  $P_{50}$  values at the two distinct temperatures. Catecholamine release thresholds, calculated on the basis of arterial blood oxygen-saturation (expressed as  $\text{CaO}_2/[\text{Hb}]$ ), were similar at both temperatures and were approximately equal to 53–55 % Hb  $\text{O}_2$ -saturation.

The results support the contention that the lowering of blood oxygen content/saturation rather than  $P_{\text{O}_2}$  *per se* is the proximate stimulus/signal causing catecholamine release in rainbow trout during acute hypoxia.

### Introduction

In response to severe environmental hypoxia, fish release the catecholamines adrenaline and noradrenaline into the circulation (Butler *et al.* 1978; Boutilier *et al.* 1988;

Key words: *Oncorhynchus mykiss*, hypoxia, adrenaline, noradrenaline, catecholamines, temperature, stress.

Metcalf and Butler, 1989; Ristori and Laurent, 1989; Aota *et al.* 1990; Fievet *et al.* 1990; Kinkead *et al.* 1991; Perry *et al.* 1991; Kakuta and Murachi, 1992; Perry and Reid, 1992; Thomas *et al.* 1992). The elevation of plasma catecholamine levels during hypoxia initiates a series of compensatory physiological processes directed towards the enhancement of branchial oxygen transfer and blood oxygen transport (see reviews by Perry and Wood, 1989; Randall, 1990; Thomas and Perry, 1992). These include (i) an enhancement of gill O<sub>2</sub>-diffusing capacity (Pettersson, 1983; Perry *et al.* 1985), (ii) an increase in blood oxygen-carrying capacity owing to the release of sequestered red blood cells from the spleen (Nilsson and Grove, 1974; Perry and Kinkead, 1989), and (iii) a rise in haemoglobin oxygen-binding affinity/capacity owing to activation of red blood cell Na<sup>+</sup>/H<sup>+</sup> exchange (see reviews by Jensen, 1991; Nikinmaa, 1992; Thomas and Perry, 1992). In addition, elevated levels of circulating catecholamines may help to sustain the hyperventilatory response during severe hypoxia [Aota *et al.* 1990; Randall and Taylor, 1991 (see also, review by Perry *et al.* (1992) for an opposing view on the role of catecholamines in the control of breathing in fish].

In teleosts, including the rainbow trout, the predominant source of circulating catecholamines is the chromaffin tissue contained within the walls of the posterior cardinal vein at the level of the anterior (head) kidney (see review by Randall and Perry, 1992). Several physiological stimuli appear to trigger the mobilization of catecholamines from the chromaffin tissue. These include release of the neurotransmitter acetylcholine from preganglionic cholinergic fibres of the sympathetic nervous system (Nilsson *et al.* 1976; Perry *et al.* 1991) and direct local effects of altered blood chemistry (Opdyke *et al.* 1983; Hathaway *et al.* 1989; Perry *et al.* 1991). During hypoxia in Atlantic cod (*Gadus morhua*) and presumably rainbow trout, catecholamines are released in response to both sympathetic (neural) stimulation and the local effects of blood hypoxaemia on the chromaffin cells (Perry *et al.* 1991). Owing to the obligate relationship between P<sub>O<sub>2</sub></sub> and blood oxygen content, it has proved difficult to distinguish between the effects of these two variables on promoting catecholamine release during hypoxia. Consequently, there is considerable uncertainty concerning the relative contributions of changes in P<sub>O<sub>2</sub></sub> and O<sub>2</sub> content in both the neural and the local control of catecholamine release (Fievet *et al.* 1990; Perry and Reid, 1992). Recently, it was suggested that the lowering of blood oxygen content is the proximate signal causing catecholamine release during hypoxia (Perry and Reid, 1992). This suggestion was based on uniform thresholds for catecholamine release in rainbow trout or American eel (*Anguilla rostrata*) when these thresholds were expressed on the basis of arterial O<sub>2</sub> content (CaO<sub>2</sub>) rather than PaO<sub>2</sub>. However, the study of Fievet *et al.* (1990) implies a specific effect of P<sub>O<sub>2</sub></sub>, despite the inherent difficulties in distinguishing the possible specific effects of O<sub>2</sub> content *versus* O<sub>2</sub> partial pressure.

In the present study, we have attempted to determine the precise nature of the stimulus causing catecholamine release during hypoxia. The experimental design was to manipulate the intrinsic properties of Hb O<sub>2</sub>-binding in trout and to assess the impact on the dynamics of catecholamine release during acute hypoxia. The Hb O<sub>2</sub>-binding affinity was altered by acclimating fish to either 5 or 15 °C. If catecholamines are indeed released into the circulation at a particular and variable PaO<sub>2</sub> threshold corresponding to a critical

and uniform reduction in O<sub>2</sub> content (Perry and Reid, 1992), then the predicted effect of acclimating fish to lower water temperature would be a lowering of the  $P_{aO_2}$  threshold at which catecholamines are mobilized, in agreement with the increase in Hb O<sub>2</sub>-affinity induced by low temperature.

## Materials and methods

### *Experimental animals*

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] weighing between 200 and 300 g (experimental  $N=116$ ) were obtained from Linwood Acres Trout Farm (Campbellcroft, Ontario) and were transported in hyperoxic water to the University of Ottawa. Fish were maintained on a 12 h:12 h L:D photoperiod in large fibreglass aquaria supplied with flowing, aerated and dechlorinated City of Ottawa tap water ( $[Na^+]=0.10\text{ mmol l}^{-1}$ ,  $[Cl^-]=0.15\text{ mmol l}^{-1}$ ,  $[Ca^{2+}]=0.35\text{--}0.40\text{ mmol l}^{-1}$ ,  $[K^+]=0.03\text{ mmol l}^{-1}$ , pH 7.7–8.0). Fish were maintained at 12 °C for approximately 2 weeks before being separated into two different temperature acclimation groups; one group was acclimated to 5 °C and the other to 15 °C. The temperature was varied by 0.5 °C per day until the desired final temperature was reached. Fish were maintained under the final acclimation conditions for at least 2 months before experimentation. Trout were fed daily to satiation using a diet of commercial trout pellets; food was withheld for 48 h prior to experimentation.

### *Animal preparation*

Fish were anaesthetized in a 0.1 g l<sup>-1</sup> solution (5 or 15 °C) of ethyl-*m*-aminobenzoate (MS 222; Sigma Chemical Company), which was adjusted to pH 7.5 with NaHCO<sub>3</sub>, and then placed onto an operating table to allow continuous retrograde irrigation of the gills with anaesthetic solution. To permit periodic blood sampling, an indwelling cannula was implanted into the dorsal aorta (Soivio *et al.* 1975) using flexible polyethylene tubing (Clay-Adams PE 50; internal diameter 0.580 mm, outer diameter 0.965 mm). Trout were revived on the operating table by irrigation of the gills with aerated water, then transferred to individual opaque acrylic experimental chambers (volume 3 l) supplied with aerated flowing water of appropriate temperature, where they were allowed to recover from the effects of anaesthesia and surgery for at least 48 h before experimentation commenced. Cannulae were flushed daily with freshwater teleost saline containing 50 i.u. ml<sup>-1</sup> ammonium heparin (Sigma Chemical Company).

### *Experimental protocol*

Individual groups of fish ( $N=5\text{--}8$  in each group) were acutely exposed to levels of hypoxia ranging between 4.0 and 10.7 kPa (30–80 mmHg). Specifically, the levels of hypoxia utilized were 10.7, 9.3, 8.0, 6.7, 6.0, 5.3, 4.7 and 4.0 kPa (80, 70, 60, 50, 45, 40, 35 and 30 mmHg). The lower limits of 4.0 kPa (30 mmHg; 5 °C) and 4.7 kPa (35 mmHg; 15 °C) were selected on the basis of the results of preliminary experiments showing marked mortality at more severe levels of hypoxia (especially at the higher temperature). In the present study, mortalities were observed at 4.7 kPa (1 fish) and 4.0 kPa (2 fish) in

the fish acclimated at 15 °C and 5 °C, respectively; the results from these animals have not been incorporated. The upper limit of 10.7 kPa (80 mmHg) was selected on the basis of the preliminary results showing a lack of catecholamine mobilization at this level of hypoxia and the level immediately preceding it (9.3 kPa/70 mmHg).

Hypoxia was initiated by first stopping the air and water flow to the experimental chamber and then quickly re-establishing water flow at the same rate using hypoxic water exiting a water/gas equilibration column previously set to the target  $P_{\text{wO}_2}$ . The  $P_{\text{wO}_2}$  was adjusted by manipulating the rates of nitrogen (Air Products Inc.) and water flow through the column. The water flow rate into the experimental chamber was always in excess of  $51 \text{ min}^{-1}$  and was sufficient to achieve the desired degree of hypoxia in the chamber within 5 min. Fish were returned to normoxic conditions by re-establishing normal (normoxic) water flow and aeration.

The inflowing water and the water within the experimental chamber were continuously monitored for  $P_{\text{O}_2}$ . Usually, the  $P_{\text{wO}_2}$  of the inflow and chamber did not vary by more than 0.2–0.3 kPa and the  $P_{\text{wO}_2}$  of the experimental chamber, rather than the column, was used in calculating the mean  $P_{\text{wO}_2}$  of the various hypoxic groups.

Blood samples (0.6 ml) were withdrawn from the dorsal aortic cannula pre-hypoxia, at 5 and 15 min after reaching the desired level of hypoxia, and 15 min after return to normoxic conditions. The arterial blood was analyzed immediately after sampling to determine  $P_{\text{aO}_2}$ , oxygen content ( $\text{CaO}_2$ ), whole-blood pH (pHa) and haemoglobin concentration ([Hb]). The remaining blood was centrifuged and the plasma (200–250  $\mu\text{l}$ ) stored at  $-80^\circ\text{C}$ , for no longer than 2 weeks, prior to determination of catecholamine levels. The red cell pellet was resuspended in teleost saline and reinjected into the dorsal aorta.

#### *Analytical procedures*

Whole-blood pH was determined with a microcapillary pH electrode (Radiometer G299A). Blood or water  $P_{\text{O}_2}$  was measured using  $P_{\text{O}_2}$  electrodes (Radiometer E5046) housed in thermostatted cuvettes (ambient water temperature 5 or 15 °C).  $P_{\text{wO}_2}$  from the equilibration column or the experimental chamber was monitored continuously by allowing water to flow by siphon through the measuring chambers of the electrodes. The pH and  $P_{\text{O}_2}$  electrodes were maintained at the appropriate acclimation water temperature and utilized in conjunction with Radiometer PHM-71 acid–base analyzers and BMS3 Mk2 blood micro-systems. The  $P_{\text{O}_2}$  electrodes were calibrated with water equilibrated to known  $P_{\text{O}_2}$  values using air-saturated water. The pH electrode was calibrated using precision buffers (Radiometer).  $\text{CaO}_2$  was measured on 20  $\mu\text{l}$  samples according to an established method (Tucker, 1967) using a Radiometer  $P_{\text{O}_2}$  electrode in a sealed chamber maintained at 37 °C. [Hb] measurements were performed in duplicate on 20  $\mu\text{l}$  blood samples using a commercial spectrophotometric haemoglobin assay kit (Sigma Chemical Company).

Plasma adrenaline and noradrenaline levels were determined on alumina-extracted samples using high performance liquid chromatography (HPLC) with electrochemical detection (Woodward, 1982). 3,4-Dihydroxybenzylamine hydrobromide (DHBA) was used as an internal standard in all determinations.

*Calculations and statistical analysis**In vivo oxygen dissociation curves*

Oxygen dissociation curves were constructed using the measured values of  $P_{aO_2}$ ,  $CaO_2$  and  $[Hb]$  from samples withdrawn pre-hypoxia and at 5 and 15 min of hypoxia; the values obtained after the return to normoxia were not utilized because of the possibility of persistent alterations of Hb  $O_2$ -affinity initiated by catecholamines. To adjust for differences in  $[Hb]$  and variable quantities of physically dissolved  $O_2$  in the plasma, the amount of haemoglobin-bound  $O_2$  per unit haemoglobin ( $[O_2]/[Hb]$ ), in  $\text{mol } O_2 \text{ mol}^{-1} \text{ Hb}$ , was calculated using  $O_2$  solubility coefficients for trout plasma (Boutilier *et al.* 1984). Oxygen dissociation curves were constructed by plotting  $[O_2]/[Hb]$  as a function of  $P_{aO_2}$  and fitting the data to a sigmoidal function using an iterative curve-fitting function in a commercial graphics software package (Sigmaplot 5.0; Jandel Scientific). Blood oxygen-affinity ( $P_{50}$ ; the  $P_{aO_2}$  at half-maximal Hb  $O_2$ -saturation) and Hill coefficients were derived from the Hill plot.

*Catecholamine release thresholds*

The  $P_{aO_2}$  and  $[O_2]/[Hb]$  thresholds for catecholamine release were calculated as described by Perry and Reid (1992). The technique is used to estimate the point at which plasma catecholamine levels rise significantly above the baseline values. First, the mean baseline catecholamine levels were calculated by incorporating all values above a critical  $P_{aO_2}$ , the criterion for which was that plasma catecholamine levels were stable for at least 10 mmHg below this value. Next, the highest individual  $P_{aO_2}$  value with a catecholamine level statistically higher than baseline (outside the 95 % confidence interval) was determined and the mean  $P_{aO_2}$  was calculated for all  $P_{aO_2}$  values below that value. The  $P_{aO_2}$  threshold was then calculated as that mean  $P_{aO_2}$  plus its 95 % confidence interval.  $[O_2]/[Hb]$  thresholds were calculated in a similar fashion.

*Statistical analysis*

Where appropriate, data are presented as mean values  $\pm 1$  standard error of the mean. The results have been statistically analyzed by analysis of variance followed by Fisher's LSD test for multiple comparisons; 95 % was accepted as the level of difference.

**Results***Blood acid-base/respiratory variables*

The effects of exposure to acute graded hypoxia on arterial blood respiratory/acid-base variables at the two acclimation temperatures are shown in Tables 1 (5 °C) and 2 (15 °C). For clarity, only the pre-hypoxic and 15 min hypoxic data are presented as there were no significant differences in any of the measured variables between 5 and 15 min of hypoxia. After 15 min of recovery from hypoxia, all of the measured blood respiratory/acid-base variables had returned to pre-hypoxic levels except for whole-blood pH (pHa) at the two severest levels of hypoxia (4.7 and 5.3 kPa  $P_{wO_2}$ ) in the 15 °C acclimated fish (data not shown).

Table 1. Arterial blood respiratory variables in rainbow trout (*Oncorhynchus mykiss*) acclimated to 5 °C before (Pre) and after 15 min of exposure to graded levels of external hypoxia ranging between 4.0 and 10.7 kPa (30–80 mmHg; nominal final  $P_{wO_2}$  values)

	Normoxia		10.7 kPa		9.3 kPa		8.0 kPa		6.7 kPa		6.0 kPa		5.3 kPa		4.7 kPa		4.0 kPa	
	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min
$P_{wO_2}$ (kPa)	21.21 (0.04)	20.92 (0.23)	21.20 (0.05)	11.05* (0.27)	21.28 (0.04)	9.18* (0.07)	21.24† (0.03)	7.97* (0.09)	21.13 (0.04)	6.58* (0.04)	20.75 (0.17)	5.75* (0.04)	20.62 (0.4)	5.29* (0.23)	21.21† (0.13)	4.55* (0.04)	21.28 (0.04)	4.23* (0.19)
$P_{aO_2}$ (kPa)	15.75† (0.72)	14.91 (1.09)	12.86† (0.84)	6.78* (1.08)	16.01† (0.59)	6.50* (0.80)	16.52† (0.24)	4.46* (0.85)	16.37† (0.63)	3.54*† (0.61)	14.78 (0.76)	2.38* (0.32)	15.32 (0.85)	2.19* (0.23)	15.08 (1.18)	1.70*† (0.11)	13.86 (2.27)	1.84* (0.15)
$CaO_2$ (vol%)	7.6 (0.5)	7.3 (0.6)	6.9 (0.5)	6.6 (0.2)	7.5 (0.4)	5.7*† (0.4)	6.8 (0.4)	5.1*† (0.7)	9.1† (0.4)	5.1*† (0.4)	8.1 (0.6)	4.0* (0.4)	7.6 (0.7)	4.8*† (0.5)	7.2 (0.3)	3.1*† (0.4)	7.7 (0.4)	3.1* (0.2)
[Hb] (g 100 ml <sup>-1</sup> )	7.4 (0.8)	6.7 (0.6)	7.2 (0.5)	6.8 (0.3)	7.4 (0.5)	6.7 (0.3)	6.5 (0.6)	5.9 (0.5)	9.2† (0.5)	8.1* (0.5)	7.1 (0.6)	6.2 (0.4)	6.7 (0.8)	6.5 (0.7)	6.8 (0.4)	7.0 (0.4)	7.0 (0.5)	6.0* (0.2)
[O <sub>2</sub> ]/[Hb] (mol mol <sup>-1</sup> )	3.12 (0.07)	3.24 (0.10)	2.89 (0.14)	2.80 (0.14)	3.03 (0.15)	2.53*† (0.12)	3.21 (0.23)	2.65*† (0.20)	2.95 (0.10)	1.92*† (0.11)	3.42† (0.10)	2.15† (0.09)	3.45 (0.18)	2.12*† (0.12)	3.15 (0.18)	1.41*† (0.10)	3.30 (0.13)	1.50* (0.08)
pHa	7.94† (0.02)	7.91 (0.01)	7.93 (0.08)	8.00† (0.04)	8.09† (0.05)	8.14† (0.05)	7.96 (0.04)	7.93 (0.09)	8.06† (0.03)	7.95 (0.10)	7.91 (0.03)	7.90 (0.06)	8.09† (0.05)	8.05† (0.08)	8.00† (0.06)	7.82*† (0.06)	8.04 (0.04)	7.72* (0.07)

Values shown are means ± 1 s.e. (in parentheses);  $N=5-8$  in each group.

\* indicates a significant difference from the pre-hypoxia values; † indicates a significant difference from the corresponding value at 15 °C.

Table 2. Arterial blood respiratory variables in rainbow trout (*Oncorhynchus mykiss*) acclimated to 15 °C before and after 15 min of exposure to graded levels of external hypoxia ranging between 4.7 and 10.7 kPa (35–80 mmHg; nominal final  $P_{wO_2}$  values)

	Normoxia		10.7 kPa		9.3 kPa		8.0 kPa		6.7 kPa		6.0 kPa		5.3 kPa		4.7 kPa	
	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min	Pre	15 min
$P_{wO_2}$ (kPa)	19.66 (0.17)	19.80 (0.05)	20.67 (0.19)	10.53* (0.19)	20.68 (0.19)	9.42* (0.15)	19.88† (0.20)	7.10* (0.25)	20.07 (0.24)	6.46* (0.37)	20.28 (0.33)	5.72* (0.21)	19.88 (0.24)	4.88* (0.15)	19.38† (0.43)	4.42* (0.12)
$P_{aO_2}$ (kPa)	11.31† (1.04)	13.22 (0.86)	15.04† (0.39)	6.70* (0.57)	13.93† (0.77)	6.04* (0.94)	14.08† (0.74)	3.88* (0.28)	13.01† (1.01)	2.35*† (0.20)	12.21 (1.32)	2.53* (0.15)	13.33 (0.73)	2.37* (0.23)	12.18 (1.20)	2.37* (0.24)
$C_{aO_2}$ (vol%)	6.7 (0.4)	6.8 (0.6)	7.2 (0.2)	6.1* (0.5)	6.1† (0.5)	4.4*† (0.5)	6.0 (0.4)	3.4*† (0.2)	7.3† (0.6)	2.7*† (0.5)	7.5 (0.5)	3.9* (0.4)	7.2 (0.5)	2.3*† (0.3)	6.6 (0.4)	1.6*† (0.1)
[Hb] (g 100 ml <sup>-1</sup> )	7.1 (0.5)	6.6 (0.5)	7.6 (0.2)	6.9* (0.2)	6.6 (0.4)	6.7 (0.3)	5.9 (0.6)	5.1 (0.6)	7.3† (0.5)	7.0 (0.4)	8.2 (0.3)	8.4† (0.2)	7.1 (0.5)	7.1 (0.5)	7.4 (0.4)	6.5 (0.6)
[O <sub>2</sub> ]/[Hb] (mol mol <sup>-1</sup> )	2.71 (0.09)	2.68 (0.06)	2.80 (0.08)	2.59 (0.10)	2.71 (0.08)	1.94*† (0.07)	3.09 (0.11)	1.83*† (0.12)	2.92 (0.09)	1.18*† (0.10)	2.71† (0.08)	1.38*† (0.05)	3.09 (0.13)	0.94*† (0.06)	2.53 (0.08)	0.77*† (0.05)
pH <sub>a</sub>	7.81† (0.02)	7.85 (0.02)	7.93 (0.02)	7.89† (0.02)	7.85† (0.02)	7.91† (0.03)	7.88 (0.02)	7.88 (0.03)	7.85† (0.02)	7.89 (0.03)	7.92 (0.03)	7.86 (0.04)	7.83† (0.02)	7.57*† (0.07)	7.78 (0.03)	7.43*† (0.06)

Values shown are means ± 1 s.e. (in parentheses); N=5–8 in each group.  
\* indicates a significant difference from the pre-hypoxia values; † indicates a significant difference from the corresponding value at 5 °C.

Exposure of fish to hypoxia caused reductions in  $P_{aO_2}$ ,  $CaO_2$  and  $O_2$  bound to haemoglobin ( $[O_2]/[Hb]$ ) that were roughly proportional to the severity of the hypoxia. The changes in  $CaO_2$  and  $[O_2]/[Hb]$  were more pronounced in the fish acclimated to 15 °C (compare Tables 1 and 2). Blood  $[Hb]$  was essentially unaltered during the hypoxia; the decreases in  $[Hb]$  that were occasionally observed (see Tables 1 and 2) were probably attributable to the blood sampling rather than being a consequence of hypoxia *per se*. Whole-blood pH remained constant at  $P_{wO_2}$  values above 5.3 kPa (40 mmHg) and 4.7 kPa (35 mmHg) in the 15 °C and 5 °C fish, respectively. Below these levels of  $P_{wO_2}$ , whole-blood pH declined significantly (Tables 1 and 2); the reduction in pHa was more pronounced in the 15 °C acclimated fish.

Whole-blood pH was consistently (although not always) higher in the 5 °C acclimated fish in accordance with the predicted inverse relationship between blood temperature and pHa (see Heisler, 1984). Occasionally, the pre-hypoxia values of  $P_{aO_2}$ ,  $CaO_2$ ,  $[Hb]$  or  $[O_2]/[Hb]$  were significantly different between the two acclimation groups, although no obvious pattern was evident.

#### *Blood oxygen dissociation curves*

*In vivo*  $O_2$  dissociation curves (Fig. 1) were constructed for the 5 and 15 °C acclimated fish using the blood respiratory data gathered during the acute hypoxia experiments (Tables 1 and 2); the recovery data were not used. In theory, the  $[O_2]/[Hb]$  of fully saturated haemoglobin is 4 mol  $O_2$  mol<sup>-1</sup> Hb. In the present study, the  $[O_2]/[Hb]$  at maximal binding was 3.2 mol mol<sup>-1</sup>, indicating approximately 83 % saturation. This value is somewhat lower than those reported for trout haemoglobin in previous studies (95–100 %; Hooton and Randall, 1967; Eddy, 1976) and indicates a significant fraction of non-functional haemoglobin of unknown origin. The  $[O_2]/[Hb]$  at maximal binding was identical at both temperatures, thereby allowing valid comparison between the two experimental groups. The apparent  $P_{50}$  values were determined assuming that maximal binding occurred at a  $[O_2]/[Hb]$  value of 3.2 mol  $O_2$  mol<sup>-1</sup> Hb. To permit valid comparisons with previous studies, however, the Hb  $O_2$ -saturation catecholamine release thresholds were calculated assuming that 100 % saturation corresponded to 4 mol  $O_2$  mol<sup>-1</sup> Hb.

At 5 °C, the *in vivo*  $P_{50}$  value was 1.9 kPa (14.0 mmHg) whereas at 15 °C the  $P_{50}$  value was increased to 3.6 kPa (26.7 mmHg). The Hill coefficients ( $n_H$ ) were 1.29 and 1.76 at 5 and 15 °C, respectively.

#### *Plasma catecholamine levels*

Regardless of acclimation temperature, plasma catecholamine levels were elevated at  $P_{wO_2}$  values less than or equal to 8.0 kPa (60 mmHg; Fig. 2). Adrenaline was the sole catecholamine released into the circulation at  $P_{wO_2}=8.0$  kPa (60 mmHg). Below 8.0 kPa (60 mmHg), both adrenaline and noradrenaline levels were elevated, with adrenaline being the prevalent circulating catecholamine in most instances (Fig. 2). At  $P_{wO_2}$  values of 5.3 kPa (40 mmHg) and 4.7 kPa (35 mmHg), plasma adrenaline levels (Fig. 2A) were significantly greater in the 15 °C acclimated fish. At  $P_{wO_2}$  values of 6.7 kPa and below,



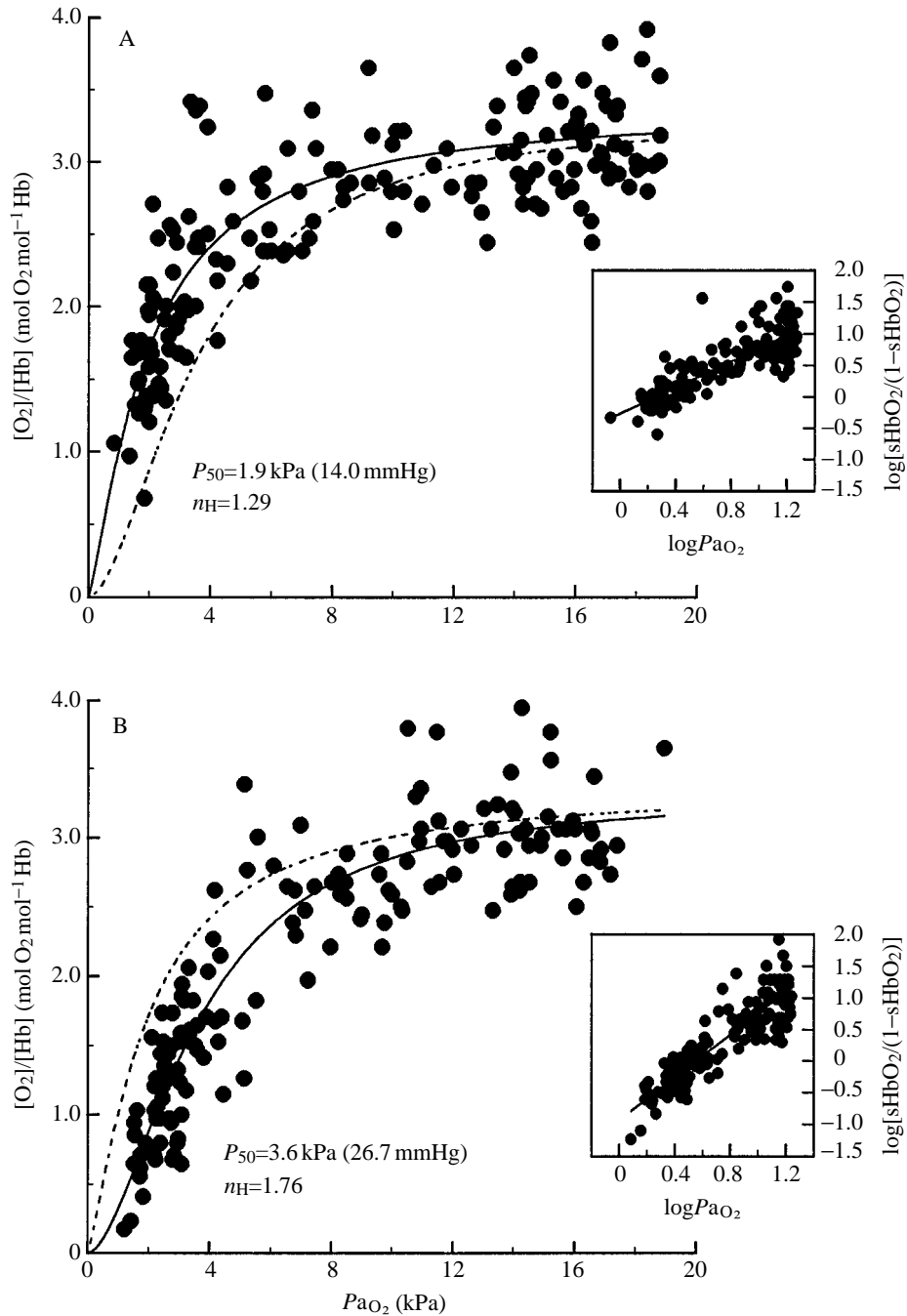


Fig. 1. *In vivo* blood oxygen dissociation curves of rainbow trout (*Oncorhynchus mykiss*) acclimated to (A) 5°C or (B) 15°C. The curves were constructed by sampling dorsal aortic blood from normoxic and acutely hypoxic fish. For comparison, the dashed lines represent the oxygen dissociation curves for the other temperature. The insets in each panel are Hill plots from which the  $P_{50}$  values were calculated. See text for further details.

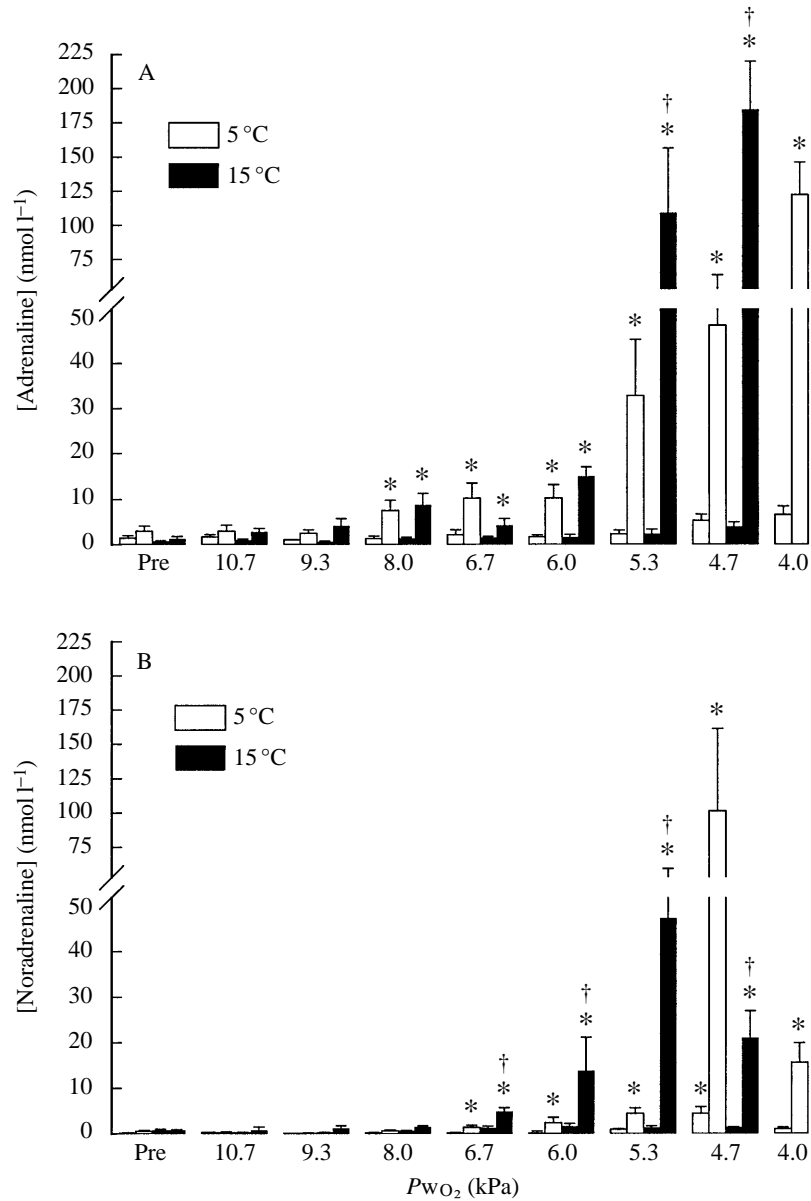


Fig. 2. The effects of graded acute (15 min) external hypoxia on (A) plasma adrenaline and (B) plasma noradrenaline levels in rainbow trout acclimated either to 5°C (open histograms) or 15°C (shaded histograms). Plasma catecholamine levels are expressed as a function of the nominal values for the partial pressure of oxygen in the water ( $P_{wO_2}$ ). The actual mean measured  $P_{wO_2}$  values did not differ significantly from the nominal values and are shown in Tables 1 and 2 for 5°C and 15°C, respectively. Values shown are means  $\pm$  1 s.e. ( $N=5-8$  for each group). \* indicates a significant difference from the pre-hypoxia value (Pre); † indicates a significant difference from the corresponding value at the other acclimation temperature.

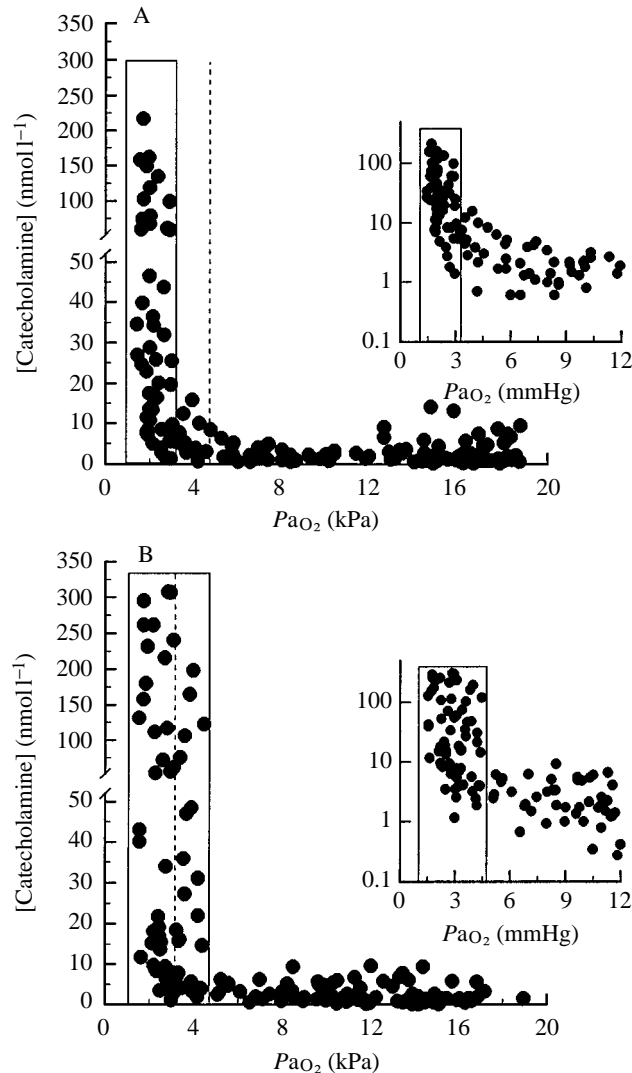


Fig. 3. The relationships between arterial blood  $P_{O_2}$  ( $P_{aO_2}$ ) and total plasma catecholamine levels ([adrenaline plus noradrenaline]) in rainbow trout acclimated to (A) 5 °C or (B) 15 °C and subjected to acute (15 min) graded hypoxia. The areas within the boxes represent the zones of catecholamine release, which were determined by calculating the  $P_{aO_2}$  thresholds for release (see Materials and methods) with the right-hand edge of the box representing the  $P_{aO_2}$  threshold of release. At each acclimation temperature, the catecholamine data have also been expressed using a logarithmic scale (insets) to show more clearly the scatter about the baseline values. At 5 °C, the  $P_{aO_2}$  release threshold for catecholamine release was 3.3 kPa (24.5 mmHg), whereas at 15 °C the  $P_{aO_2}$  threshold was 4.6 kPa (34.5 mmHg). The dashed line in each panel represents the release threshold at the other acclimation temperature. See text for further details.

plasma noradrenaline levels (Fig. 2B) were always higher (with the exception of 4.7 kPa) in the 15 °C fish.

Fig. 3 illustrates the relationships between  $P_{aO_2}$  and the total plasma catecholamine levels (adrenaline plus noradrenaline). At each acclimation temperature, plasma catecholamine concentrations remained remarkably constant over a wide range of  $P_{aO_2}$  but then increased abruptly when a critical  $P_{aO_2}$  threshold was reached. The  $P_{aO_2}$  thresholds were widely different at the two acclimation temperatures. At 5 °C, the calculated  $P_{aO_2}$  threshold for catecholamine release was 3.3 kPa (24 mmHg; Fig. 3A), whereas at 15 °C the corresponding  $P_{aO_2}$  threshold was 4.6 kPa (34.5 mmHg; Fig. 3B). The difference in these catecholamine release  $P_{aO_2}$  thresholds (1.3 kPa) was approximately equal to the difference in the *in vivo*  $P_{50}$  values (1.7 kPa) at the two distinct temperatures.

The relationships between  $[O_2]/[Hb]$  and plasma catecholamine levels are shown in Fig. 4. The calculated catecholamine release thresholds were similar at each acclimation temperature, varying only between 2.2 (5 °C) and 2.1 (15 °C) mol  $O_2$  mol<sup>-1</sup> Hb, corresponding to 53–55 % Hb  $O_2$ -saturation.

#### *Water–blood $P_{O_2}$ relationships*

The relationships between  $P_{wO_2}$  and  $P_{aO_2}$  during normoxia and hypoxia are illustrated in Fig. 5.  $P_{aO_2}$  declined with decreasing  $P_{wO_2}$  in an essentially similar manner in both acclimation groups. During moderate hypoxia ( $P_{wO_2} > 8.0$  kPa), for any given value of  $P_{wO_2}$ ,  $P_{aO_2}$  at 5 °C was generally higher than that at 15 °C. These differences were not apparent at the more severe levels of hypoxia.

### **Discussion**

#### *Effects of acclimation temperature on catecholamine release*

Recently, Perry and Reid (1992) proposed a mechanism to explain the abrupt release of catecholamines into the circulation during exposure of teleost fish to environmental hypoxia. According to this theory, catecholamines are mobilized from the chromaffin tissue as the blood oxygen content is lowered to a critical catecholamine release threshold. Further, the theory predicts that the blood  $P_{O_2}$  at which this threshold is reached will vary according to the affinity of Hb  $O_2$ -binding. Thus, fish possessing blood of high Hb  $O_2$ -affinity (low  $P_{50}$ ) would be expected to release catecholamines at considerably lower blood  $P_{O_2}$  values than fish with low Hb  $O_2$ -affinity (high  $P_{50}$ ). In each instance, however, catecholamine levels would rise at a uniform value of Hb  $O_2$ -saturation corresponding to 45–60 % Hb  $O_2$ -saturation. This model was based on data gathered from two species possessing widely different Hb  $O_2$ -affinities, the American eel (*Anguilla rostrata*) and the rainbow trout (*Oncorhynchus mykiss*). As appreciated by the authors (Perry and Reid, 1992), it was conceivable that the differences in the catecholamine release profiles in eel and trout simply reflected intrinsic differences in  $P_{O_2}$  release thresholds and that the uniformity of the  $O_2$  content release thresholds may have been coincidental. The present study was designed to test this model by using a single

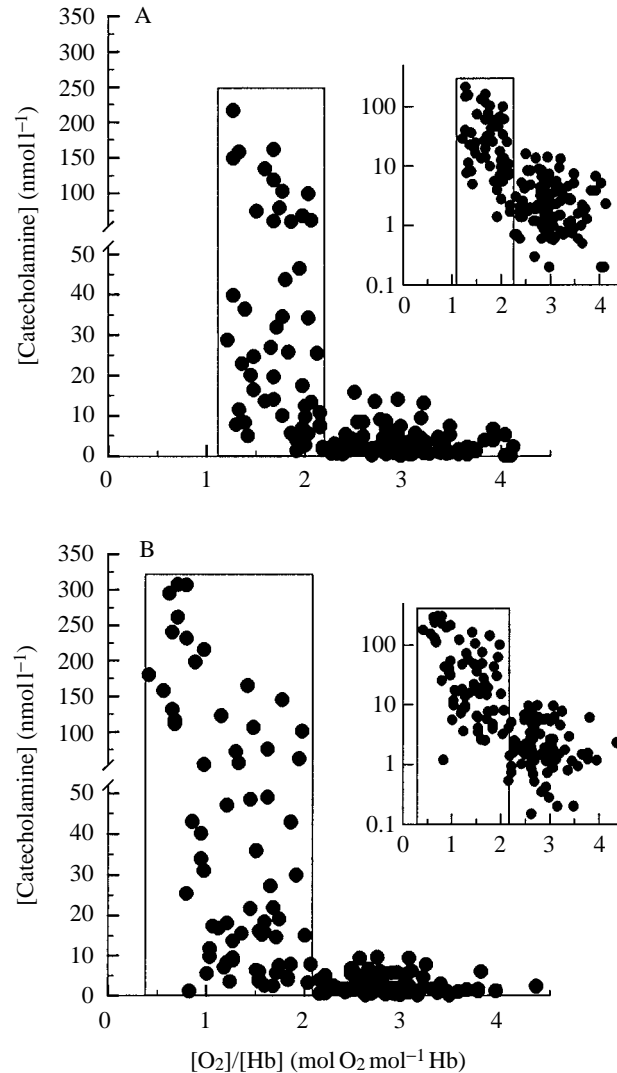


Fig. 4. The relationships between arterial blood O<sub>2</sub> content (expressed as [O<sub>2</sub>]/[Hb]) and total plasma catecholamine levels ([adrenaline plus noradrenaline]) in rainbow trout acclimated to (A) 5 °C or (B) 15 °C and subjected to acute (15 min) graded hypoxia. The areas within the boxes represent the zones of catecholamine release, which were determined by calculating the [O<sub>2</sub>]/[Hb] thresholds for release (see Materials and methods), with the right-hand edge of the box representing the [O<sub>2</sub>]/[Hb] threshold of release. At each acclimation temperature, the catecholamine data have also been expressed using a logarithmic scale (insets) to show more clearly the scatter about the baseline values. At 5 °C, the release threshold for catecholamine release was 2.18 mol mol<sup>-1</sup>, whereas at 15 °C the corresponding threshold was 2.09 mol mol<sup>-1</sup>. See text for further details.

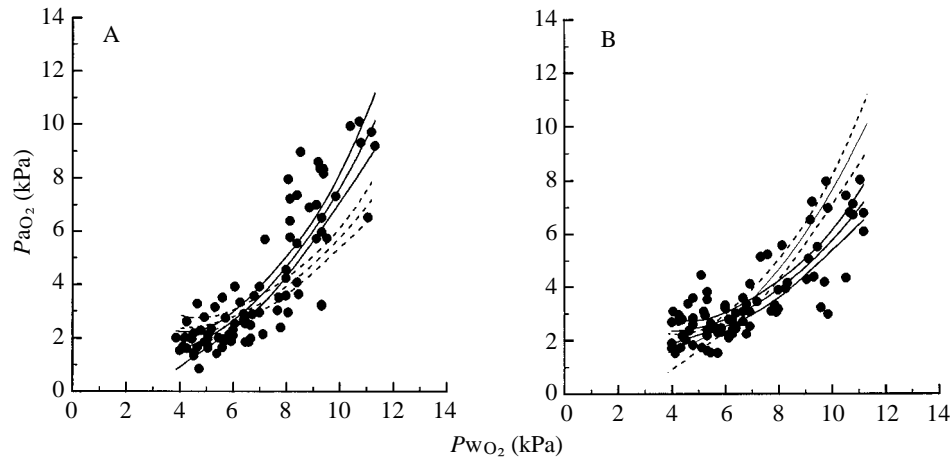


Fig. 5. The relationship between water  $P_{O_2}$  ( $P_{wO_2}$ ) and arterial blood  $P_{O_2}$  ( $P_{aO_2}$ ) during normoxia or graded acute (15 min) in rainbow trout (*Oncorhynchus mykiss*) acclimated to either (A) 5 °C or (B) 15 °C. For comparison, the dashed lines represent the  $P_{wO_2}$ – $P_{aO_2}$  relationships for the other acclimation temperature. The curves ( $\pm 95\%$  confidence intervals) were fitted using a commercial graphics software package (Sigmaplot 5.0; Jandel Scientific).

species (rainbow trout) acclimated to different temperatures as a means to modify Hb  $O_2$ -affinity experimentally (see below).

The results of this study support the mechanism proposed by Perry and Reid (1992). At each acclimation temperature, catecholamines were released into the circulation as the blood oxygen status traversed a critical  $P_{O_2}$  threshold corresponding to a uniform value of approximately 53–55 % Hb  $O_2$ -saturation. This catecholamine release threshold was similar to the release threshold (45–60 % Hb  $O_2$ -saturation) reported in a previous study (Perry and Reid, 1992). Owing to the decrease in  $P_{50}$  in the 5 °C acclimated fish, this threshold was reached at a lower  $P_{aO_2}$  than in the 15 °C acclimated fish. In other words, the difference in the  $P_{aO_2}$  thresholds at the two temperatures was essentially equal to the difference in the  $P_{50}$  values. The simplest explanation for these data is that the lowering of Hb  $O_2$ -saturation (or a closely related variable such as blood  $O_2$  content) is the proximate signal causing the release of catecholamines rather than a lowering of blood  $P_{O_2}$  *per se*. Regardless of the mechanism underlying the relationship between blood  $O_2$  content and catecholamine release, the obvious and important consequence of this relationship is that catecholamines are released into the circulation only upon marked impairment of blood  $O_2$  transport. Unlike the ventilatory and cardiovascular adjustments to hypoxia (see reviews by Perry *et al.* 1992; Fritsche and Nilsson, 1993), which often begin with only slight reductions in  $P_{wO_2}$ , the release of catecholamines is not initiated until  $P_{wO_2}$  is lowered to very low levels [usually below 8 kPa (60 mmHg)] (see review by Randall and Perry, 1992). Given that the predominant effect of elevated catecholamine levels is to enhance branchial  $O_2$  transfer and blood  $O_2$  transport, the physiological significance of their delayed release into the circulation after only a severe reduction of blood  $O_2$  content is evident. From a design viewpoint, it would be impractical to link

changes in  $P_{O_2}$  *per se* to catecholamine mobilization because  $P_{O_2}$  is *not* a reliable indicator of blood  $O_2$  content. This reflects the non-linear relationship between  $P_{O_2}$  and Hb  $O_2$ -saturation as well as the interactive effects of numerous allosteric modifiers of Hb  $O_2$ -binding, such as  $CO_2$ ,  $H^+$  and organic phosphates (Weber and Jensen, 1988; Jensen, 1991). Thus, the reliance of catecholamine release on a critical lowering of blood  $O_2$  content may have evolved so as to improve  $O_2$  delivery when it is compromised by a developing hypoxaemia.

A linkage between blood  $O_2$  content and Hb  $O_2$ -saturation and plasma catecholamine levels has been suggested by the results of previous studies in addition to the aforementioned comparison of trout and eel (see above). First, anaemic fish release catecholamines into the circulation (Iwama *et al.* 1987; Perry *et al.* 1989) even under hyperoxic conditions (Perry *et al.* 1989). In anaemic fish, Hb  $O_2$ -saturation is not lowered, which suggests that there is a specific role for the lowering of blood  $O_2$  content in causing release. Second, it was shown that the cause of catecholamine release during hypercapnic acidosis in trout is the associated hypoxaemia (owing to the Root effect) rather than the acidosis itself (Perry *et al.* 1989). Third, Fievet *et al.* (1990) reported that the  $Pa_{O_2}$  threshold for catecholamine release in trout was substantially lowered after repeated episodes of acute hypoxia. Our own interpretation of these data is that Hb  $O_2$ -affinity was raised after the initial episode of hypoxia [a result, at least in part, of catecholamine release (Nikinmaa, 1983)] and thus led to a lowering of the  $Pa_{O_2}$  threshold. Although Fievet *et al.* (1990) alluded to a controlling role of blood  $O_2$  tension in catecholamine release, the underlying reasons for this assumption are unclear. Indeed, to our knowledge, there are no data in the literature supporting a specific/direct role of blood  $P_{O_2}$  in catecholamine release in fish.

Catecholamine release is not the only physiological process that appears to be associated with blood  $O_2$  content levels in fish. Numerous studies have implicated the reduction of blood  $O_2$  content as a key variable controlling ventilation (see reviews by Randall, 1982; Shelton *et al.* 1986), whereas there is considerably more uncertainty as to the specific role of blood  $P_{O_2}$ .

#### *Effects of acclimation temperature on Hb $O_2$ -affinity*

The advantages and disadvantages of determining  $P_{50}$  values from *in vivo*  $O_2$  dissociation curves have been discussed in detail previously (Perry and Reid, 1992). Briefly, the merit of the *in vivo*  $O_2$  dissociation curve is that it yields functional  $P_{50}$  values that encompass the net effects of potential curve modifiers, including changes in blood acid-base status and elevated catecholamine levels. The  $P_{50}$  values reported in the present study are similar to those previously reported *in vitro* (Milligan and Wood, 1987; Soivio *et al.* 1980; Vorger, 1986) or *in vivo* (Tetens and Christensen, 1987) values. The reasons for the significant amount of non-functional haemoglobin (Hb  $O_2$ -saturation was only 83 % at maximal binding) in the fish used in the present are unknown, but it presumably reflects an unusually large fraction of methaemoglobin.

The results of the present study demonstrated an increase in Hb  $O_2$ -affinity as temperature was lowered from 15 to 5 °C [ $P_{50}$  decreased from 3.6 kPa (26.7 mmHg) to 1.9 kPa (14.0 mmHg)]. Although the inverse relationship between temperature and Hb

O<sub>2</sub>-affinity is well documented following acute changes in blood temperature (e.g. Vorger, 1986; see also reviews by Weber and Jensen, 1988; Jensen, 1991), considerably less is known of the chronic effects of acclimation to different temperatures. It is generally believed, however, that chronic temperature changes elicit smaller effects on Hb O<sub>2</sub>-affinity than do acute changes (see review by Wood, 1980). For example, Weber *et al.* (1976) demonstrated that acclimation of rainbow trout to temperatures varying between 5 and 22 °C for as long as 4 months was without effect on the Hb O<sub>2</sub>-affinity of whole blood (as assessed *in vitro*). Clearly, the results of the present study showing an effect of chronic acclimation on Hb O<sub>2</sub>-affinity are in marked contrast to the study of Weber *et al.* (1976). Further, the changes in *P*<sub>50</sub> were essentially similar to the changes that accompany acute temperature changes *in vitro* (e.g. Vorger, 1986). Red blood cell intracellular pH (red blood cell pH<sub>i</sub>) or levels of red blood cell organic phosphates were not measured in the present study, making it difficult to compare these results with those of previous studies. Although not always statistically significant, whole-blood pH (pH<sub>a</sub>) was generally elevated in the fish acclimated to 5 °C (compare Tables 1 and 2) in accordance with the usual inverse relationship between pH<sub>a</sub> and temperature (Heisler, 1984). Thus, it is also likely that red blood cell pH<sub>i</sub> was elevated in the 5 °C acclimated fish given that hydrogen ions are passively distributed across the red blood cell membrane (see Nikinmaa, 1992).

#### *Other potential causes/correlates of catecholamine release*

Exposure of fish to the more severe levels of hypoxia (4.0–5.3 kPa; 30–40 mmHg) elicited marked acidosis of the blood (Tables 1 and 2). It is tempting to speculate, therefore, that the blood acidosis is a cause (or one of the causes) of the greatly elevated plasma catecholamine levels during severe hypoxia. Although several studies have reported significant correlations (Tang and Boutilier, 1988; Perry and Reid, 1992) or relationships (Boutilier *et al.* 1986; this study) between the extent of blood acidosis and circulating catecholamine levels, it is difficult to ascribe a *direct* role to blood acidosis in causing catecholamine release because of the hypoxaemia that normally accompanies acidosis in teleost fish. Furthermore, it has been demonstrated (Perry *et al.* 1989; Aota *et al.* 1990) that acidosis itself does not initiate catecholamine release in trout unless it is associated with blood hypoxaemia. An additional problem in assigning a role for blood pH changes in the control of catecholamine release is the inherent difficulty in separating the cause of release from the consequences of release. Catecholamines, when released into the blood, cause acidification of the plasma as a result of stimulation of red blood cell Na<sup>+</sup>/H<sup>+</sup> exchange (see reviews by Nikinmaa, 1992; Thomas and Perry, 1992) and thus high levels of catecholamines would normally accompany blood acidification even if acidosis itself were not a cause of catecholamine release. Finally, at moderate levels of hypoxia (>5.3 kPa; 40 mmHg), blood acidosis is clearly not a factor in triggering release because blood pH is either unaltered at such times (this study) or even elevated owing to hyperventilation. Variations in the *P*aO<sub>2</sub> versus *P*wO<sub>2</sub> relationship also cannot explain the differing patterns of release at the different temperatures, as these relationships were essentially indistinguishable at the *P*wO<sub>2</sub> levels at which catecholamines are released (Fig. 5).



The results of this and other studies (Perry *et al.* 1989; Thomas *et al.* 1992; Perry and Reid, 1992) provide compelling evidence that lowering of blood Hb O<sub>2</sub>-saturation and/or blood oxygen content is the factor that signals catecholamine release during hypoxia. However, owing to the nature of this study in which acclimation to different temperatures was used as a tool to modify Hb O<sub>2</sub>-affinity, we cannot exclude the involvement of other temperature-dependent factors such as thermal modulation of a P<sub>O<sub>2</sub></sub> receptor. The important theme that is emerging from these studies is that catecholamines are released into the circulation of fish only when a critical threshold of blood O<sub>2</sub> content is reached. Further experiments are required to elucidate fully the mechanisms linking depression of blood O<sub>2</sub> content to the mobilization of catecholamines from chromaffin tissue.

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